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TITLE: Development of a Blood-Based Biomarker Panel for Indeterminate Lung Nodules

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Lung cancer screening with low-dose CT (LDCT) has been shown to reduce mortality by 20%, although there are concerns including high false positivity, cost, and radiation exposure. Blood-based markers are a promising and attractive approach to complement LDCT because of the potential to identify those subjects that need to undergo further work-up for their indeterminate nodules. The main objective of my proposed project is to develop a blood-based biomarker panel from three platforms (protein, autoantibody and microRNA) to distinguish malignant lung nodules from benign lung nodules. We have so far assayed 20 protein markers, 122 autoantibodies, 20 microRNAs, and a metabolite DAS, in the 149 malignant lung nodules and 187 benign lung nodules. Though preliminary, the combined panel yielded AUC of 0.838, which was significantly higher than the AUC of pro-SFTPB and DAS (AUC = 0.722, P = 0.0025), suggesting potential usefulness of our biomarker panel in interpretation of indeterminate lung nodules. During the next period, we will develop and validate a biomarker panel for nodule diagnosis with integrating LDCT imaging. Assuming a prevalence of malignant nodule 3.6% as observed in NLST trial leads to specificity ≥ 35% for non-malignant nodules subjects, sparing 1/3 of unnecessary work-ups with high confidence that they are indeed lung cancer free.

15. SUBJECT TERMS

Nothing listed

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1. Introduction

Lung cancer screening with low-dose CT (LDCT) has been shown to reduce mortality by 20%, although there are concerns including high false positivity, cost, and radiation exposure. Blood-based markers are a promising and attractive approach to complement LDCT because of the potential to identify those subjects that need to undergo further work-up for their indeterminate nodules. We have identified through a series of discovery and initial validation studies, a set of protein and autoantibody biomarkers with potential for early detection. In this proposed study, we will integrate circulating microRNAs, which have recently emerged as additional promising biomarkers for lung cancer, with our protein and autoantibody biomarkers and develop a blood-based biomarker panel to reduce unnecessary and invasive work-up by determining among screened subjects those individuals with lung nodules who would need or would not need further diagnostic work-up, and consequently to reduce mortality associated with lung cancer through more effective screening strategies.

2. Keywords

Lung cancer, diagnosis, biomarker, protein, autoantibody, microRNA, low-dose CT screening

3. Accomplishments

Major goals

Major goals #1: protein, autoantibody and microRNA profiling of plasmas from subjects with malignant lung nodules and subjects with benign nodules from the PanCan set.

Milestones:

- IRB/HRPO approval (1-5 months): 100%
- Profiling of protein, autoantibody and microRNA markers in the PanCan set (6-10 months): 100%

Major goal #2: selection of biomarkers and development of an optimal model in the PanCan set.

Milestone:

- Development of an optimal model in the PanCan set (11-13 months): 50%

Accomplishment

The main objective of my proposed project is to develop a blood-based biomarker panel from three platforms (protein, autoantibody and microRNA) in samples collected in a screening setting from the Pan-Canadian Early Detection of Lung Cancer Study (PanCan) and test the marker combination in an independent screening cohort, the German Lung Cancer Screening Intervention Trial (LUSI), to distinguish malignant lung nodules from benign lung nodules. The markers from the three platforms will be subjected to model building and the added benefit of combining across platforms will be scrutinized. An optimal model will be built, integrating known lung cancer risk factors, findings from LDCT. For the proposed studies, we have obtained access to sets of samples from two independent cohorts: The Pan-Canadian Early Detection of Lung Cancer Study (PanCan) and The

German Lung Cancer Screening Intervention Trial (LUSI). In addition to these samples, we recently obtained access to additional set of plasma samples from the UT Southwestern in collaboration with Dr. Adi Gazdar, consisting of 52 malignant lung nodules and 35 benign lung nodules (the UTSW set). These additional samples will provide us further opportunities to refine and validate our biomarker panel.

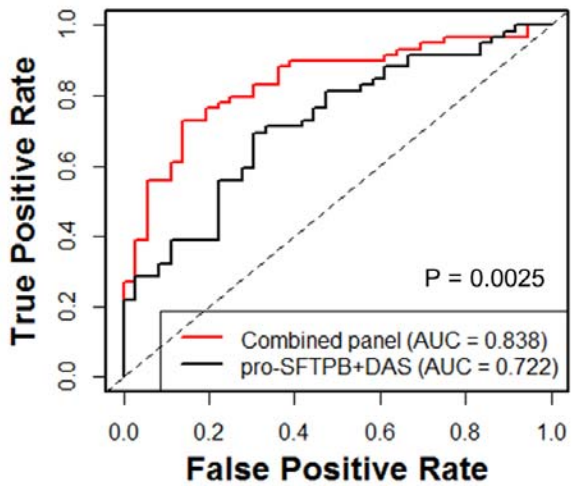
We have so far assayed 20 protein markers, 122 autoantibodies, 20 microRNAs, and a metabolite DAS, which we recently identified as a prediagnostic lung cancer biomarker (Wikoff et al. JCO 2015), in the 115 malignant lung nodules and 196 benign lung nodules in the UTSW and PanCan sets for nodule diagnosis (Table 1).

While we are still blinded to the results of PanCan samples and we have not finalized panel development, we preliminarily examined if combination of biomarkers would improve the performance of pro-SFTPB and DAS in discriminating malignant lung nodules from benign lung nodules. The combined panel yielded AUC of 0.838, which was significantly higher than the AUC of pro-SFTPB and DAS (AUC = 0.722, P = 0.0025) in the UTSW set (Figure 1), suggesting potential usefulness of our biomarker panel in interpretation of indeterminate lung nodules.

Table 1. Characteristics of subjects.

	UTSW set	PanCan set
Total (n)	87	224
Sex (male/female)	47/40	107/117
Smoking status (Current/Former/Never)	55/24/8	80/125/19
Nodule (malignant/benign)	52/35	63/161

Figure 1. Performance of combined biomarker panel in the UTSW set.



Opportunities for training and professional development

The proposed project provided me an excellent opportunity to present our biomarker studies at the Interdisciplinary Translational Education and Research Training Program (ITERT), which the Department of Translational Molecular Pathology (TMP) has offered at MD Anderson Cancer Center since 2016. ITERT is a multidisciplinary training program for graduate students, postdoctoral and M.D. fellows, aiming 1) to provide a broad and comprehensive education covering timely concepts in translational cancer research, including risk factors, early detection, molecular-targeted therapy, disease monitoring and mechanism of recurrence, as well as computational and systems biology; and 2) to provide an innovative environment, infrastructure, faculty, career-, and administrative support to allow trainees to develop innovative translational cancer research skills; and 3) to promote a sense of community and an interactive research and educational environment among trainees and faculty in the TMP.

Opportunities for training and professional development provided to people who worked on the project.

A postdoctoral fellow (Dr. Tanaka) and a research assistant (Ms. Gargy) are working on this project. Through one-on-one training as well as technical seminars, they improved their skills including protein and autoantibody assay development, analytical assessment of the assays, isolation and assay of microRNA, and statistical analysis of biomarker candidates.

Dissemination of the results to communities

Nothing to Report.

Plan in the next reporting period

During the next period, we will develop and validate a biomarker panel for nodule diagnosis with integrating LDCT imaging. To increase the number of samples, the assay results of UTSW and PanCan set will be standardized and combined. Biomarker panel will be developed with integrating clinical factors and findings from LDCT imaging. Then we will adjust its threshold to correspond to 98% sensitivity for malignant nodules and test its specificity against benign nodules. The biomarker/imaging panel will be tested in the LUSI set.

Regarding LDCT imaging, volumetric analysis and textural analysis of lung nodules will be performed to help distinguish benign from the malignant lung nodules, in collaboration with the expert radiologists Drs. Jeremy Erasmus and Myrna Godoy at MDACC. Since we aim at “rule out” low risk people from invasive/expensive work-up they otherwise would be subjected to by current medical practice, the decision role will require a high sensitivity for true positive nodules, i.e. lung cancer, and a NPV for people whose biomarker value is below threshold. Therefore Major Goal #3 is to validate a threshold from a biomarker/imaging panel that has NPV > 99.8%, sensitivity ≥ 98% in the LUSI set. Assuming a prevalence of malignant nodule 3.6% as observed in NLST trial leads to specificity ≥ 35% for non-malignant nodules subjects, sparing 1/3 of unnecessary work-ups with high confidence that they are indeed lung cancer free.

4. Impact

Distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project

Although a large number of lung cancer biomarker studies have been published, the vast majority of lung cancer biomarker studies consist of one type of biomarkers being evaluated, whether consisting of circulating proteins, autoantibodies, microRNAs or other types without an appreciation of their performance relative to other markers. Therefore there is the need to assess and compare the relative contribution of different biomarkers and assay platforms side by side using the same sample sets, in order to determine their comparative performance and the contribution of individual markers to a robust panel. Although it is still preliminary and need to be validated in an independent sample set, our blood-based biomarker panel integrating protein, autoantibody, microRNA, and metabolite, significantly improved the performance in discriminating malignant lung nodules from benign lung nodules compared to the performance of pro-SFTPB and DAS, suggesting the potential of this innovative approach to develop an optimal blood-base biomarker panel with integrating multiple assay platforms.

The impact on other disciplines

Nothing to Report.

The impact on technology transfer

Nothing to Report.

The impact on society beyond science and technology

Nothing to Report.

5. Changes/Problems

Nothing to Report.

6. Products

Nothing to Report.

7. Participants & Other Collaborating Organizations

Individuals in the project
Ayumu Taguchi: No change.
Ignacio Wistuba: No change.
Samir Hanash: No change.
Ziding Feng: No change.

Name:	Gargy Parhy
Project Role:	Research Assistant
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	4
Contribution to Project:	Ms. Parhy will work on assay development and validation under supervision of Drs. Ayumu Taguchi and Ichidai Tanaka.
Funding Support:	The MDACC startup funds

Name:	Ichidai Tanaka
Project Role:	Postdoctoral Fellow
Researcher Identifier (e.g. ORCID ID):	NA
Nearest person month worked:	3
Contribution to Project:	Dr. Tanaka is highly experienced in assay development and analysis and will work on assay of biomarkers and marker validation under supervision of Dr. Ayumu Taguchi.
Funding Support:	The MDACC startup funds

Change in the active other support of the PD/PI(s) or senior/key personnel
Nothing to Report.

8. Special Reporting Requirements
NA

9. Appendices
NA